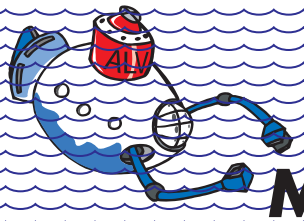

Message in the Bottles

Focus
Estimating primary productivity

Grade Level
9-12 (Earth Science/Chemistry)

Focus Question
How can we measure primary productivity in water bodies?

Learning Objectives
Students will be able to identify the three realms of the Arctic Ocean, and describe the relationships among these realms.

Students will be able to explain the relationships between gross primary productivity, net primary productivity, and respiration.

Students will understand how oxygen production and consumption can be measured and used to estimate primary productivity in water bodies.

Materials
- ❒ "Light-Dark Bottle Activity Guide," one for each student group
- ❒ Dissolved oxygen test kits (e.g., LaMotte kit #5860), one kit for each student group OR dissolved oxygen meter with stirring probe
- ❒ Water sample bottles (e.g., LaMotte #0688-DO or equivalent; one bottle is included with each test kit, but you will need to order additional bottles for this activity), three per student group; one of the three bottles should be covered with aluminum foil to make it opaque
- ❒ Carboy or other large container(s) for water collected from local water body, fitted with siphon to allow students to withdraw samples without aerating the water
- ❒ Appropriate incubation space (see "Learning Procedure" Step 2)

Audio/Visual Materials
None

Teaching Time
Two or three 45-minute class periods

Seating Arrangement
Groups of 3-6 students

Maximum Number of Students
Unlimited, depending upon physical space and financial resources for materials

Key Words
Pelagic
Benthic
Sympagic
Primary productivity
Gross primary productivity
Net primary productivity
Respiration
Autotroph
Light-dark bottle

Background Information
The Arctic Ocean is the smallest of the world's four ocean basins with a total area of about 5.4 million square miles or 14 million square kilometers (roughly 1.5 times the size of the

United States). It is bordered by Greenland, Canada, Alaska, Norway, and Russia. The Arctic Ocean has the widest continental shelf of any ocean, extending 750 mi (1,210 km) from the coast of Siberia, but also has areas that are quite deep. The average depth is 12,000 ft (3,658 m) and the maximum depth is 17,850 ft (5,441 m). The Chukchi Sea provides a connection with the Pacific Ocean via the Bering Strait, but this connection is very narrow and shallow, so most water exchange is with the Atlantic Ocean via the Greenland Sea.

The floor of the Arctic Ocean is divided by three submarine ridges (Alpha Ridge, Lomonosov Ridge, and the Arctic Mid-Oceanic Ridge) one of which (the Lomonosov Ridge) creates a relatively isolated area known as the Canadian Basin. This area is particularly interesting to scientists because its isolation could mean that it contains unique life forms that are found nowhere else on Earth. But the Arctic Ocean is not easily explored: it is almost entirely covered with ice for eight months of the year, a drifting polar ice pack covers the central and western portions year-round, and sea temperature seldom rises above 0°C. Although the Arctic is still the world's least explored ocean, new expeditions are about to give us much greater knowledge of the mysteries of this polar frontier.

At this point, we know that there are at least three distinct biological communities in the Arctic Ocean. The Sea-Ice Realm includes plants and animals that live on, in, and just under the ice that floats on the ocean's surface. Because only 50% of this ice melts in the summer, ice flows can exist for many years and can reach a thickness of more than six ft (2 m). Sea ice is not usually solid like an ice cube, but is riddled with a network of tunnels called brine channels that range in size from microscopic (a few thousandths of a millimeter) to more than an inch in diameter. Diatoms and algae inhabit these channels and obtain energy from sunlight to produce biological material through photosynthesis. Bacteria, viruses, and fungi also inhabit the channels, and together with diatoms and algae provide an energy source (food) for flatworms, crustaceans, and other animals. This community of organisms is called sympagic, which means "ice-associated." Partial melting of sea ice during the summer months produces ponds on the ice surface that contain their own communities of organisms. Melting ice also releases organisms and nutrients that interact with the ocean water below the ice.

The Pelagic Realm includes organisms that live in the water column between the ocean surface and the bottom. Melting sea ice allows more light to enter the sea, and algae grow rapidly since the sun shines for 24 hours a day during the summer. These algae provide energy for a variety of floating animals (zooplankton) that include crustaceans and jellyfishes. Zooplankton, in turn, is the energy source for larger pelagic animals including fishes, squids, seals, and whales.

When pelagic organisms die, they settle to the ocean bottom, and become the energy source for inhabitants of the Benthic Realm. Sponges, bivalves, crustaceans, polychaete worms, sea anemones, bryozoans, tunicates, and ascidians are common members of Arctic benthic communities. These animals provide energy for bot-

tom-feeding fishes, whales, and seals.

Most of our knowledge about biological communities in the Arctic Ocean comes from studies on portions of the ocean near the continental shelves. Very little research has been done on the sea ice, pelagic, and benthic realms in the deepest parts of the Arctic Ocean. These areas are the focus of the 2002 Ocean Exploration Program's Arctic Ocean Expedition.

The foundation of all biological communities are autotrophic ("self-nourishing") organisms that are able to synthesize organic material from simpler inorganic substances using an external source of energy. This process is known as primary production or primary productivity. Photosynthetic autotrophs (green plants) use sunlight as their energy source, while chemosynthetic organisms (such as bacteria found around hydrothermal vents) obtain energy from chemical compounds. In both cases, organic material produced by autotrophs becomes a source of energy and raw materials for many other organisms. In the Arctic Ocean, more than 50% of the average primary productivity comes from single-celled algae that live near the ice-seawater junction, and this interface is critical to the polar marine ecosystem. Researchers on the Arctic Ocean Expedition plan to measure primary productivity and water chemistry at various study sites to learn more about processes that support the Arctic's marine biological communities.

The procedure for measuring primary productivity by photosynthetic autotrophs is easily understood if we recall the basic equation for photosynthesis:

$6CO_2 + 6H_2O + \text{sunlight} > C_6H_{12}O_6 + 6O_2$

Primary productivity is usually defined as grams of carbon produced per square meter per day. So we need to know how much glucose ($C_6H_{12}O_6$) is produced by photosynthetic plants in a known volume of water over a specific time. The equation above shows that we can either measure glucose directly, or we could measure the amount of CO_2 consumed or the amount of O_2 produced, since these are directly related to the amount of glucose produced.

Two techniques are commonly used to measure primary productivity. The first is to measure the uptake of radioactive carbon (14C). A known volume of water is placed in a clear glass bottle, and a known quantity of radioactive carbon dioxide is added to the sample. The bottle is placed in sunlight for a fixed period of time, and then filtered. The organic material produced by photosynthesis will be trapped on the filter, and can be measured by measuring the amount of radioactivity on the filter. This is the method used by researchers on the Arctic Ocean Expedition.

A key point with this technique is to remember that plants consume organic material through respiration as well as produce organic material through photosynthesis. So the organic material on the filter is the total amount of organic material produced MINUS the amount of organic material consumed by the plants themselves. This is called NET primary productivity. If we want to know the TOTAL or GROSS primary productivity, we need to know how much organic material was consumed by respiration.

To measure total primary productivity, researchers often use the light-dark bottle technique. With this method, changes in dissolved oxygen concentration are used to measure photosynthesis and respiration (since oxygen is produced in photosynthesis and consumed in respiration). Water samples are placed into clear glass bottles, and a duplicate sample is placed into bottles that are painted black or covered with tape so that no light can reach the sample. Without light, no photosynthesis can occur. Respiration, however, will continue to take place. A third sample is prepared in a clear glass bottle. Dissolved oxygen in the third sample is measured with a dissolved oxygen meter or chemical methods, and establishes the initial dissolved oxygen present in the light and dark bottle samples at the beginning of the experiment.

The bottles are stoppered, and allowed to incubate for 30 minutes to 24 hours (depending upon the expected level of productivity; more productive waters require shorter incubation times). The bottles may be incubated in the water body from which the samples were collected (*in situ* incubation), or they may be incubated in the laboratory (*in vitro* incubation). The advantage of *in situ* incubation is that the samples are exposed to natural levels of light and temperature, and the result probably gives the best estimate of actual productivity in the water body. The advantages of *in vitro* incubation is that it may be more practical in many cases, and is easier to do in a normal class period.

After the incubation period is completed, dissolved oxygen is measured in all bottles. Oxygen is expected to have increased in the light bottles due to photosynthesis, and to have decreased in the dark bottles due to respiration. When the measurements are completed, total oxygen produced is calculated by adding the oxygen consumed in the dark bottles to the oxygen produced in the corresponding light bottles. Total oxygen consumption can be used to calculate gross primary production as explained below.

In this activity, students will measure gross and net primary productivity in a local water body. This activity is best done in late spring or early fall when primary production is still fairly high. Except in vary warm areas, primary productivity is generally low during winter months, and may be difficult to measure. The following procedure uses a chemical titration method to measure dissolved oxygen as it is much less expensive than electronic oxygen measuring instruments (which typically cost more than $1,000). If a dissolved oxygen meter is available, visit http://www.epa.gov/OWOW/monitoring/vol.html for suggestions on appropriate procedures.

Learning Procedure

1. Review Background Information on the Arctic Ocean and its three known biological realms. Emphasize that the three realms are coupled, and that photosynthesis by microscopic algae (phytoplankton) provides the energy for other organisms in these realms (i.e., the algae are the "base of the food web"). You may want to mention that other marine systems (such as those in the vicinity of hydrothermal vents) are not dependent on photosynthesis for energy, but rely on

chemosynthesis instead (see http://oceanexplorer.noaa.gov for lesson plans and background information on these systems). If necessary, review the basic concepts of photosynthesis. Be sure students understand that photosynthesis can be limited if one or more of the necessary components is in limited supply. Explain how primary productivity can be measured, and be sure students understand the difference between gross and net primary productivity. You may want to have the students practice the dissolved oxygen measuring technique before beginning the light-dark bottle activity.

2. Prepare a suitable area for incubating the water samples. The area should have strong artificial illumination and relatively constant temperature. A constant-temperature water bath with two 40-watt fluorescent tubes at a distance of 50 – 100 cm is ideal. Less elaborate incubation areas can work, too. Be sure to avoid direct exposure to sunlight, and select an area in which the bottles can remain undisturbed during the incubation period.

   Prepare the water sample bottles. Each bottle should have a unique number, and one bottle for each group should be covered with aluminum foil to make the bottle opaque.

3. Obtain sufficient water from a local water body (lake, pond, river, estuary, etc.) to supply each student group with at least 300 ml of water. Try to collect the water within 24 hours of the beginning of the activity, and try to maintain light and temperature levels reasonably close to those in the area from which the water was collected. Set up the supply container(s) with siphons, and avoid shaking or vigorously aerating the water since it will be impossible to measure an increase due to photosynthesis if the water becomes saturated with oxygen.

4. Have each student group follow procedures given in the "Light-Dark Bottle Activity Guide" Steps 1 – 3.

   Be sure students understand the importance of avoiding excessive aeration during transfer of the water from the supply container to the sample bottles.

5. After the samples have incubated for 24 hours, have students complete Steps 4 – 6 in the "Light-Dark Bottle Activity Guide."

6. Have students present their results to the entire class. Lead a discussion interpreting these results. If different groups obtain very different results, discuss possible reasons. These should include natural variability among samples as well as experimental error.

   Be sure students understand the basis for the numbers used in calculating gross productivity, net productivity, and respiration. Units of measure are very important in these calculations, and can be confusing.

The following discussion provides some additional background for those who want to discuss the basis for the calculations in more detail. The LaMotte titrator provides a direct read-out of dissolved oxygen concentration in

parts-per-million (ppm), which is equivalent to mg/kg and also to mg/l for freshwater. Seawater has a slightly higher density than freshwater.

Once oxygen production or consumption has been calculated in mg O_2/liter/hour, this figure can be converted to grams of carbon per liter per hour by multiplying by 0.000375:

a. 6 moles of O_2 are produced for each mole of $C_6H_{12}O_6$;
b. there are 32 grams of O_2 in each mole of O_2, and 72 grams of carbon in each mole of $C_6H_{12}O_6$;
c. so there are 6 x 32 = 192 grams of O_2 produced for every 72 grams of carbon produced, which is equivalent to 72 ÷ 192 = 0.375 grams carbon per gram O_2. Since there are 1,000 mg in a gram, one mg O_2 is equivalent to 0.000375 grams of carbon.

Since 1 liter is equivalent to 1,000 cubic centimeters, and there are 1,000,000 cubic centimeters in a cubic meter, we can determine how many grams of carbon are produced per cubic meter per hour by multiplying grams of carbon per liter per hour by 1,000.

Finally, since there are 24 hours in a day, we can convert grams of carbon per cubic meter per hour to grams of carbon per cubic meter per day by multiplying by 24.

We can combine these steps by multiplying mg O_2 /liter/hour by 9, since 0.000375 x 1,000 x 24 = 9. The final result is grams of carbon per cubic meter per day. If we wanted to express primary productivity as grams of carbon per square meter per day, we would multiply grams of carbon per cubic meter per day by the depth in meters over which there is adequate light for photosynthesis to take place. Since there is obviously more light near the surface than in deeper waters, researchers might make light-dark bottle measurements at several depths and integrate the results to calculate total productivity in the water column, or they might measure the intensity of photosynthetically-active radiation at different depths and use this information to estimate productivity in the entire water column.

The BRIDGE Connection

www.vims.edu/bridge/polar.html

The "Me" Connection

Have students write a one-page essay on the importance of marine primary productivity to their own lives. To make it a bit more challenging, narrow the question to the importance of ARCTIC marine primary productivity.

Connections to Other Subjects

English/Language Arts, Chemistry, Mathematics

Evaluation

Completion of Data Sheets included in the "Light-Dark Bottle Activity Guide" may be used as one basis for evaluation. Students may also be asked to prepare written interpretations of their data prior to or instead of the presentation and discussion in Step 6.

Extensions

1. Have different student groups incubate their samples under different light condi-

tions. Layers of gray plastic window screen can be used to reduce light reaching the light bottles.

2. Have students visit http://oceanexplorer.noaa.gov to get more details on activities and results of primary productivity studies on the Arctic Ocean Expedition.

Resources

http://oceanexplorer.noaa.gov – Find out more about the Arctic Ocean Expedition and read daily documentaries and reports of discoveries posted for your classroom use.

http://www.arctic.noaa.gov/ – NOAA's Arctic theme page with numerous links to other relevant sites.

http://maps.grida.no/arctic/ – Thematic maps of the Arctic region showing populations, ecoregions, etc.

http://www.thearctic.is/ – A web resource on human-environment relationships in the Arctic.

http://www.dfo-mpo.gc.ca/regionhs/CENTRAL/arcexplor – Website produced by Fisheries and Oceans Canada on the Arctic.

http://www.epa.gov/OWOW/monitoring/vol.html – Links to water quality monitoring techniques

National Science Education Standards

Content Standard A: Science As Inquiry

- Abilities necessary to do scientific inquiry
- Understanding about scientific inquiry

Content Standard B: Physical Science

- Chemical reactions

Content Standard C: Life Science

- Interdependence of Organisms

Content Standard D: Earth and Space Science

- Energy in the Earth system

Activity developed by Mel Goodwin, PhD, The Harmony Project, Charleston, SC

Light-Dark Bottle Activity Guide

1. Obtain three water sample bottles. One of these should be black glass or be covered with aluminum foil to make it opaque. Record the numbers of the three bottles on the Data Sheet.

2. Carefully fill each of the bottles with water from the large container. Put the end of the siphon all the way to the bottom of each bottle before releasing the clamp. Avoid shaking the sample, splashing, or anything else that will add oxygen to the sample water. Tap the bottom of the bottle gently on a table to dislodge any air bubbles.

3. Put one clear glass bottle and the opaque bottle in the incubation area. Record the time on the Data Sheet. As soon as this is done, measure the dissolved oxygen in the remaining clear glass bottle using the "Dissolved Oxygen Determination Procedure" described below. Record the results on the Data Sheet.

4. After about 24 hours, remove the two bottles from the incubation area. Record the time on the Data Sheet.

5. Measure the dissolved oxygen in each of the two bottles using the "Dissolved Oxygen Determination Procedure" described below. Record the results on the Data Sheet.

6. Calculations:
   a. Calculate the incubation time in hours and hundredths of hours (divide minutes by 60 and round to two decimal places).

   b. Calculate "Net Oxygen Production" by subtracting "Initial Dissolved Oxygen" (determined in Step 3) from "Final Dissolved Oxygen (Light Bottle)." The units for the answer will be parts-per-million O_2, which is the same as mg O_2 per liter.

c. Calculate "Oxygen Consumed by Respiration" by subtracting "Final Dissolved Oxygen (Dark Bottle)" from "Initial Dissolved Oxygen." Again, the units for the answer will be parts-per-million O_2, which is the same as mg O_2 per liter.

d. Calculate "Total Dissolved Oxygen Production" by adding "Oxygen Consumed by Respiration" to "Net Oxygen Production." Yes, you guessed it, the units for the answer will be parts-per-million O_2, which is the same as mg O_2 per liter.

e. Calculate "Total Carbon Production" by multiplying "Total Dissolved Oxygen Production" by 0.375 mg C/mg O_2. The units for the answer will be mg C per liter.

f. Calculate "Carbon Production Rate" by dividing "Total Carbon Production" by "Incubation Time." The units for the answer will be mg C per liter per hour.

g."Total Daily Productivity" is expressed in units of "grams Carbon per cubic meter, per day." To convert "Carbon Production Rate" to "Total Daily Productivity," multiply "Carbon Production Rate" by 1,000 to convert liters to cubic meters, then divide by 1,000 to convert mg to grams, then multiply by 24 to convert hours to days. You're right, you really only have to multiply by 24, since conversion to grams and cubic meters cancel each other out.

Light-Dark Bottle Data Sheet

Names of Researchers_____

Light Bottle No. ____ Dark Bottle No. ____ Initial Sample Bottle No. _____

Incubation Start Date _____ Incubation Start Time _____

Incubation End Date _____ Incubation End Time _____

Initial Dissolved Oxygen Determination

	Titration #1	Titration #2	Titration #3	Average
Beginning Titrator Reading	_____	_____	_____	_____
Final Titrator Reading	_____	_____	_____	_____
Dissolved Oxygen (ppm)	_____	_____	_____	_____

Final Dissolved Oxygen (Light Bottle) Determination

	Titration #1	Titration #2	Titration #3	Average
Beginning Titrator Reading	_____	_____	_____	_____
Final Titrator Reading	_____	_____	_____	_____
Dissolved Oxygen (ppm)	_____	_____	_____	_____

	Titration #1	Titration #2	Titration #3	Average
Beginning Titrator Reading	_____	_____	_____	_____
Final Titrator Reading	_____	_____	_____	_____
Dissolved Oxygen (ppm)	_____	_____	_____	_____

Calculations:

a. Incubation Time = _____ hours
b. Net Oxygen Production = _____ mg O_2 per liter
c. Oxygen Consumed by Respiration = _____ mg O_2 per liter
d. Total Dissolved Oxygen Production = _____ mg O_2 per liter
e. Total Carbon Production = _____ mg C per liter
f. Carbon Production Rate = _____ mg C per liter per hour
g. Total Daily Productivity = _____ grams C per cubic meter per day

Dissolved Oxygen Determination Procedure

Sample Preservation

CAUTION: The chemicals used in this procedure are caustic. Safety gloves and glasses should be worn during this procedure (see safety recommendations).

STEP 1. Remove the cap from the water sample bottle and add 8 drops of Manganous Sulfate Solution.

STEP 2. Add 8 drops of Alkaline Potassium Iodide Azide Solution.

STEP 3. Cap and mix, allow precipitate to settle.

STEP 4. Add 8 drops of Sulfuric Acid 1:1.

STEP 5. Cap and mix until reagent and precipitate dissolve. Sample is now "fixed."

NOTE: Following the completion of the Sample Preservation, contact between the water sample and the atmosphere will not affect the test result. Once the sample has been "fixed" in this manner, it is not necessary to perform the actual test procedure immediately, but the titration should be completed no more than 8 hours following fixation.

Titration Procedure

*Before titrating samples, rinse the titrator with Sodium Thiosulfate as follows: Draw a small amount (about 1/2 inch) of Sodium Thiosulfate into the titrator. Remove the titrator from the Sodium Thiosulfate container and draw the plunger back and forth several times to wet the inside of the titrator with the Thiosulfate Solution. Empty the titrator and proceed with Step 1. (This only needs to the done once, at the beginning of each analysis session.)

STEP 1. Fill titration tube to 20 ml line with "fixed" sample. Then cap.

STEP 2. Fill titrator with Sodium Thiosulfate Solution; add one drop at a time to sample. Swirl sample between each addition until color is a very faint yellow.

STEP 3. Remove titrator and cap. Add 4 drops of Starch Solution. Replace cap and titrator.

STEP 4. Titrate sample drop by drop until blue color just disappears. Do not add any more Sodium Thiosulfate than is necessary to produce the color change. Be sure to swirl the test tube after each drop.

STEP 5. Read titrator scale for result in ppm (parts per million) Dissolved Oxygen.

STEP 6. Carry out Test Procedure on a second sample from the same bottle. When re-filling the titrator, you do not have to empty any Sodium Thiosulfate remaining from the previous titration. When you are finished titrating, discard any remaining Sodium Thiosulfate. DO NOT RETURN UNUSED CHEMICALS TO THE SUPPLY CONTAINERS!

STEP 7. Record the results of the two tests on the Data Sheet to the right of the space for final reading. Perform a third test if the first two tests differ by more than 0.2 ppm.

Additional Notes

- Hold dropper bottles vertically when adding drops of Manganous Sulfate and Alkaline Potassium Iodide Solutions. These reagents are added in excess, so the precise number of drops is not critical; i.e., if you add 9 or 10 drops you do NOT have to start over. However, it is necessary to add the Manganous Sulfate first.

- The amount of sample to be titrated is CRITICAL. Measure carefully. The bottom of the meniscus should rest on top of the white line of the titration tube. (A meniscus is a curved upper surface of a liquid column that is concave when the containing walls are wetted by the liquid.)

- The titration is also extremely CRITICAL. When the amount of DO is above 10 ppm, you will have to refill the syringe. For accurate results, fill to 0 mark, then continue the titration. When the blue color just disappears, add 10 to the reading on the titrator scale. The result is dissolved oxygen concentration in parts per million.

- When and how much Starch Solution is added is not critical. The important thing is that the sample turns blue. Simply add titrant until the sample is light yellow; add Starch Solution and continue adding drops one by one, very carefully, while swirling constantly until solution just turns clear. The first disappearance of blue color is the endpoint.

- You are required to run titrations on each sample. Record the results of the two tests on the data sheet. If the amount of DO recorded for the second test is more than 0.2 ppm different from the first test, you must do a third test. Record the average of the two closest test results in the appropriate place on the data form.